

## ON THE BLIGHT OF SORGHUM.

THE tissues of the different organs of sorghum, under certain conditions which are not as yet perfectly known, may become the seat of an intense production of red pigment which impregnates them. The cells die and disintegrate, the disease being known as sorghum blight (*sorgho brûlé*, *Hirsebrand*).

The disease was first described in Italy by Palmeri and Comes,<sup>1</sup> who have attributed it to the development of Saccharomycetes and bacteria. Later, in America, Burrill (1887) studied anew the blight, and, after having isolated sporogenous bacteria from the infected tissues, he attempted the inoculation of healthy plants. The results were variable; however, the author inferred the parasitism of the *Bacillus Sorghi*, nov. spec., and prescribed some measures for the preservation of plantations from the invasion of the disease. Analogous experiments by Kellerman and Swingle<sup>2</sup>, more convincing than the preceding, because of the number of infections obtained, led to the same conclusions. More recently, Bruyning<sup>3</sup> has examined some plants of sorghum attacked by the blight, and formulated some conclusions different from those of the preceding authors. After having isolated several bacteria from the red tissues Bruyning retained two species which he considered the only factors of the fermentative disease, but these species are chromogenous outside the sorghum. These microbes acted symbiotically, superposing their respective pigments, the one yellow, the other red, the mixture giving the coloration observed in the tissues. These views were not supported by any experiment of inoculation.

I have recently studied some specimens of blighted sorghum coming from Algeria.<sup>4</sup> I was able to convince myself that in this case the phenomena of the blight were caused by the parasitic development of yeasts in the tissues of the plant.

<sup>1</sup>Notizie preliminari sopra alcuni fenomeni di fermentazione del Sorgho saccarino vivente (Accad. d. Sc. fis. e mat. di Napoli, fasc. 12, 1883).

<sup>2</sup>Report of Bot. Depart. of the Kansas Stat. Agri. Coll. 1888.

<sup>3</sup>La brûlure du Sorgho, etc., et les bactéries qui la provoquent (Arch. Néerland. 4<sup>e</sup> et 5<sup>e</sup> livr., pp. 297-330. 1898).

<sup>4</sup>These infected stems were sent to the botanical laboratory of the School of Pharmacy by Professor Trabut. Professor Guignard had the kindness to entrust me with the examination of them.

Already, by microscopic examination, it had been ascertained that in many regions of the central parenchyma of the stem the cells and the intercellular spaces enclosed masses of a small ovoid yeast, measuring from 1.5 to 2.5 $\mu$  on an average. It was, moreover, the only microorganism which could be directly observed, even with great magnification and with the aid of staining reagents.

It was easy to isolate this yeast in a pure state by taking up the germs with a sterilized needle from the center of the stem, the section being made with a flamed scalpel, or by removing small cylinders of the red tissue from the pith by means of a sterilized trocar. The first culture liquid was a 5 per cent. glucose bouillon. Some subsequent isolations by means of Petri dishes with bouillon sugar gelatin have given, to the exclusion of all other organisms, white colonies formed by a yeast morphologically identical with that which had been observed in the diseased sorghum.

Cultivated on the surface of carrot, potato, and on different gelatin or gelose media with sugar, the yeast grows slowly in creamy-white colonies. It apparently keeps the same dimensions as those observed in the tissues of the plant. Sown in unfermented grape juice, or in various artificially sweetened liquids suitable for the culture of yeast, this organism shows a feeble alcoholic fermentation. The fermenting power is slightly increased by a series of re-sowings in the same medium. My attempts to obtain ascospores have been fruitless, and I am not able at present to classify this yeast with the true *Saccharomyces*.

The mere fact of isolating a yeast from the tissues of the blighted sorghum is not sufficient to enable one to conclude that this yeast is a parasite, and that it brings about the symptoms of the disease. It is known, in fact, that a large number of these ferments may be encountered on the surface of stems and leaves; it would not be astonishing, therefore, if some dead tissues, still containing in their cells a part of their reserve sugar, were invaded by the saprophytic development of a superficial yeast. The following experiments, however, show that the yeast isolated from the diseased sorghum does develop in the healthy tissues of the plant, and in them brings about the phenomena of the blight.

Sorghum plants raised from seed and cultivated in a hothouse during the months of November and December 1898 and January 1899 were inoculated with pure cultures. All aseptic precautions were

taken to avoid the introduction of foreign microbes. I used Dr. Roux's sterilizable syringe with a fine injection needle, in order to reduce to the minimum the wound necessary for the introduction of the culture. Only the stems were inoculated. The epidermis was exposed by cutting a small flap in the leaf sheath, carefully disinfecting by means of a red-hot iron, and, after the inoculation, the opening sealed by means of warm wax or sterilized paper. The plants experimented upon were successively examined at intervals of time varying from five days to two months. In every case the yeast had developed and multiplied in the intercellular spaces, and in the interior of the living cells to a distance of 10 to 15<sup>mm</sup> above and below the point of inoculation.

The microscopical appearance was that which may be observed in the tissues of sorghum spontaneously infected; the lesion was also rendered visible in the same way by the red coloration of the parenchyma and fibrovascular bundles. These latter transfuse the coloring matter the whole length of the internode, and quite beyond the infected region, so that the appearance of the pigment at a certain point of the tissue is not a sure sign that the parasite is present at that point. On the surface of the stem may be observed long red lines corresponding to the outer bundles and their contiguous parenchyma seen through the transparent stem.

In these experiments, the isolation of the parasite from the affected pith has shown, by comparison with the control, that the parasitic yeast was the same as that in the cultures used as a starting point.

It is probable that the reserve sugars of the cells of the sorghum constitute the principal food of the parasite. Unfortunately the volume of infected tissue, limited and especially difficult to separate out, has not permitted the changes carried on by the parasite in the chemical composition of the plant to be estimated in this particular.

Are other yeasts capable of producing in the sorghum similar phenomena of parasitism? The following experiments answer in the affirmative. Inoculations have been made aseptically in the stems of healthy sorghum with pure cultures of wine yeast (round yeast of champagne [Bowzy]). The parasitism has been established under the same conditions as before, the yeast developing in the intercellular spaces and in the pith cells of the stem with the accompanying production of the characteristic red pigment, and with transfusion by the bundles of the internode.

On the other hand, what is the origin of the pigment? It is a general observation that wounds inflicted on the tissues of the sorghum developed a red coloration around the injured part. It is important to understand precisely in the preceding experiments the rôle of the local lesion induced by the inoculating needle. Aseptic punctures in the pith of healthy sorghum stems were made under conditions identical with those of the preceding experiments, the inoculating fluid alone being omitted. Under these conditions the pigment appeared in the wounded cells, but it was not abundant and was rigorously confined to the wound. The quantity of coloring matter thus produced is not capable of being carried by the bundles and of spreading beyond the actual point of the lesion. The experiment shows, however, that the chromogenous property belongs to the wounded cells of the sorghum.

From the preceding facts it may be concluded :

1. That yeasts may develop in the living cells of the sorghum.
2. That the parasitism of these yeasts may bring about an intense red coloration of the plant tissues, this coloration being the same as that which may be observed in the disease of sorghum called the *blight*. The production of the pigment appertains to the affected cells, and the parasite takes part only through the lesion which it produces.

These results confirm the old hypothesis of Palmeri and Comes, who, observing the fermentative phenomena of the red juice of the pith of the blighted sorghum, had inferred from it the parasitic action of the *Saccharomycetes* without giving it experimental proof. The same facts, moreover, are not contradictory of the experiments of Burrill, Kellerman, and Swingle. In fact, it may be concluded that, the red coloration being the result of a chromogenous function characteristic of the wounded cells of the plant, different parasites, yeasts or bacteria, may, by developing in the tissues, induce by continued lesion the production of a considerable quantity of the pigment.

On the contrary, it is necessary to make complete reservations as to the conclusions of Bruyning, who, attributing to the bacteria themselves the chromogenous function, denies to all micro-organisms lacking this function while outside the host plant the power of inducing the phenomena of sorghum blight.—MAXIME RADAIS, *School of Pharmacy, Paris*.